Supplementary Material (ESI) for Chemical Communications

Supporting Information

Distance-Dependent Quenching and Enhancing of Electrochemiluminescence from CdS:Mn Nanocrystals Film by Au Nanoparticles for highly sensitive detection of DNA

Yun Shan, Jing-Juan Xu*, Hong-Yuan Chen

Key Laboratory of Analytical Chemistry for Life Science (Ministry of Education of China), School of Chemistry and Chemical Engineering, Nanjing University, Nanjing 210093, P. R. China

Experimental Section

Materials. The Oligonucleotides (aquired from Shenggong Bioengineering Ltd Company, Shanghai, China) had the following sequences: Hairpin DNA molecular beacon probe (24-base loop and 5-bp stem): 5'-NH_2-$(CH_2)_6$-CCCGGTTGGTGTGGTTGG ATTGATCGTAGGTACAACC-$(CH_2)_6$-SH-3'. Target: 3'-CACACCAACCTAACTAGCATCCAT-5'. One-base mismatch: 3'-CACACCAACCTA ACTAGCATACAT-5'. Three-base mismatch: 3'-CACTCCAACCTGACTAGCATACAT-5'. Noncomplementary: 3'-AGATAAGCATACGACTGAGATTCA-5'. A shorter hairpin DNA molecular beacon probe (16-base loop and 2-bp stem): 5'-NH_2-$(CH_2)_6$-CCCGGTTGCAAGACGG AAAGACCCCAACC-$(CH_2)_6$-SH-3' and its target: 5'-GGGTCTTTCCGTCTTG-3'. $HAuCl_4$, 6-mercapto-1-hexanol (MCH, SH-$(CH_2)_6$-OH, >97.0%), 1-methylimidazol, 3-mercaptopropionic acid (MPA), N-(3-dimethylaminopropyl)-N'-ethyl-carbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS) were purchased from Sigma-Aldrich (St. Louis, MO). All reagents were used as received. 0.1M phosphate buffer solution (PBS) containing 0.05M $K_2S_2O_8$ (pH 8.3) was used for ECL detection, and 0.1M Tris-HCl buffer containing 0.1M NaCl (pH 7.4) for hybridization and preparation of DNA stock solutions. All aqueous solutions were prepared using ultra-pure water (Milli-Q, Millipore).

Apparatus. The electrochemical and ECL emission measurements were conducted on a MPI-A multifunctional electrochemical and chemiluminescent analytical system (Remax Electronic Instrument Limited Co., Xi'an, China) at room temperature. Both electrochemical and ECL properties were investigated by cyclic voltammetry (CV) at glassy carbon electrode (GCE, 3mm diameter). A three-electrode configuration was used where a Pt wire and SCE electrode served as the counter and reference electrodes, respectively. All the ECL emission measurements were carried out in 0.1M pH 8.3 phosphate buffer (KH_2P0_4-K_2HPO_4) using 0.05M $K_2S_2O_8$ as a coreactant. The observation window was placed in front of the photomultiplier tube biased at 500V. Transmission electron microscopy was performed with a JEOL model 2000 instrument operating at 200 kV accelerating voltage. The UV-vis absorption spectra were obtained on a Shimadzu UV-3600 UV-vis-NIR photospectrometer (Shimadzu Co.). The doped concentration of Mn in CdS NCs was determined by X-ray photoelectron spectroscopy (XPS, ESCALAB 250).

Synthesis of CdS:Mn NCs. A typical synthesis involved dissolution of $Cd(NO_3)_2 \cdot 4H_2O$ (0.1683 g) and $Mn(CH_3COO)_2 \cdot 4H_2O$ (0.0134 g) in 30 ml ultra-pure water. The reaction mixture was heated to 70ºC under stirring, to which a freshly prepared solution of Na_2S in 30 ml ultra-pure water was added and instantly orange-yellow precipitates were obtained. The reaction was held at 70ºC for 3 h with continuous refluxing. The final reaction precipitates were centrifuged and washed thoroughly with absolute ethanol two times, followed by washing with ultra-pure water to get rid of any Mn^{2+} and other ions remaining outside the clusters. The average size of the CdS:Mn NCs was about 5nm, as indicated by transmission electron microscopy (Figure S-1). The Mn concentration determined by XPS for the as-prepared CdS:Mn NCs was 1.34 atom%. (Figure S-2) For the NCs thin-film preparation, the resulting powders were ultrasonically dispersed in ultra-pure water to obtain a colloidal solution of CdS:Mn NCs, which was kept in a refrigerator at 4 ºC.

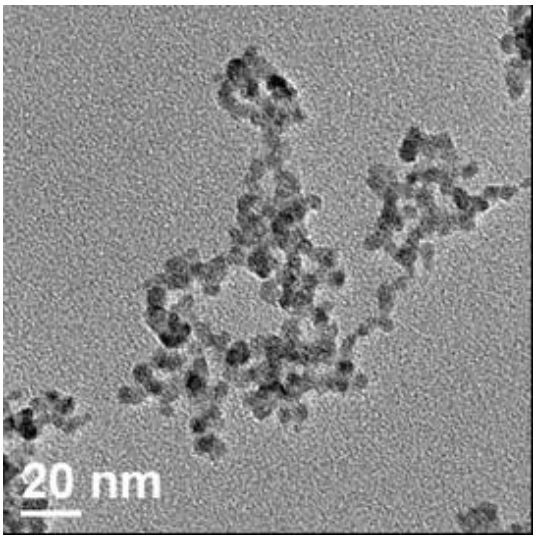
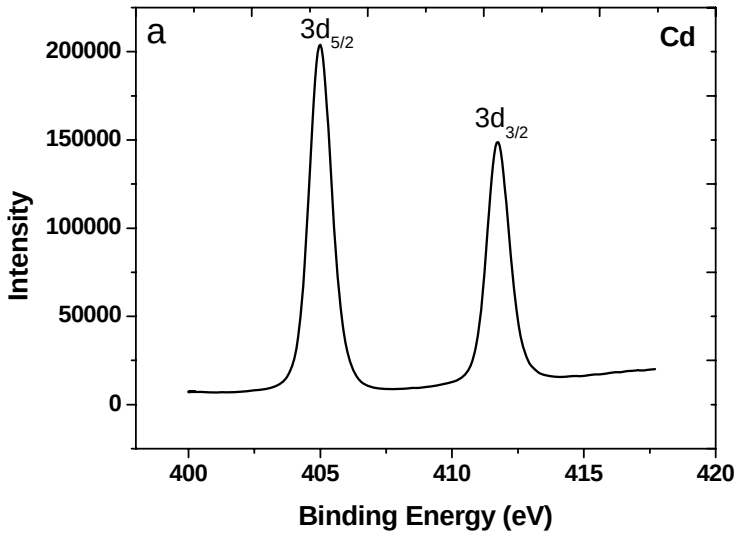


Figure S-1. TEM picture of as-synthesized CdS:Mn NCs



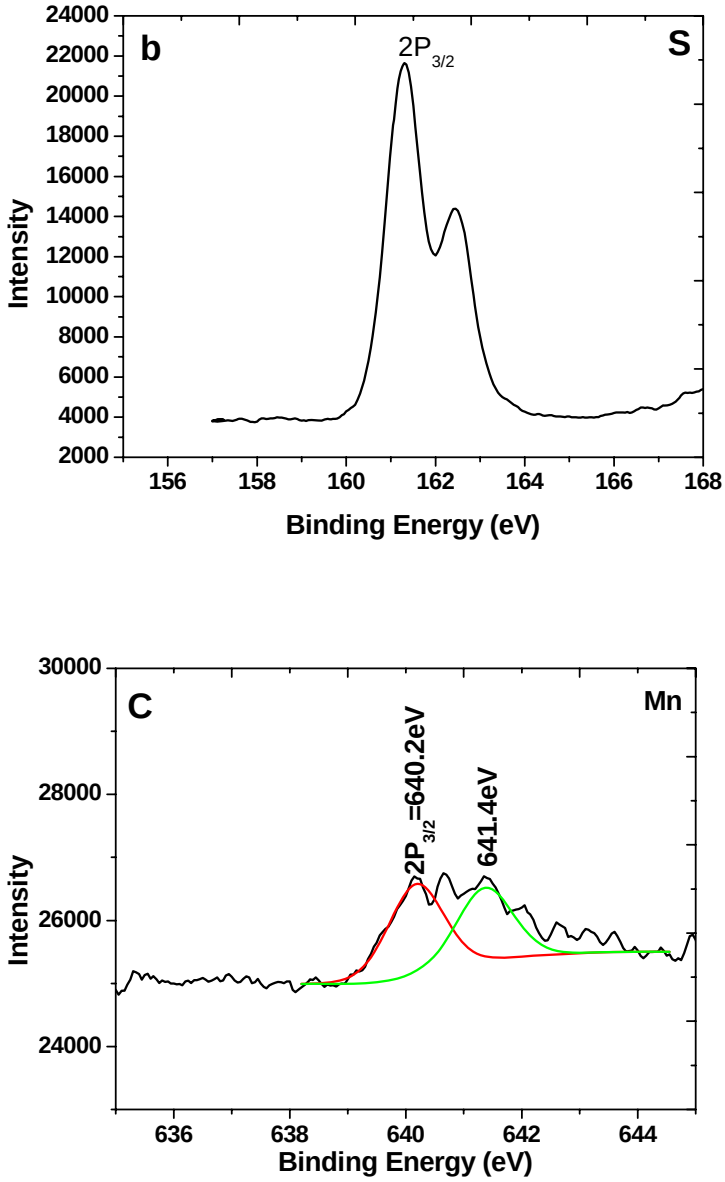


Figure S-2. XPS spectra for (a) Cd $3d_{5/2}$ and $3d_{3/2}$, (b) S $2p_{3/2}$ and (c) Mn $2p_{3/2}$ in the CdS:Mn NCs sample.

Synthesis of Uncapped Au NPs. Au NPs were synthesized according to reference S1. Briefly, 0.6 ml of ice cold 0.1 M $NaBH_4$ was added to 20 ml aqueous solution containing 2.5×10^{-4} M $HAuCl_4$ under stirring. The solution immediately turned to orange-red color, indicating the formation of gold nanoparticles. UV-Vis spectrum of the as prepared AuNPs is shown in figure S-3. The colloidal AuNPs solution exhibited an absorption maximum ca. 525 nm corresponding to the surface plasmon band (Figure S-3). The average particle size measured from TEM was 5±1 nm (Figure S-4). These particles were stored at 25 ºC for 3 h and then kept in a refrigerator at 4 ºC for further use.

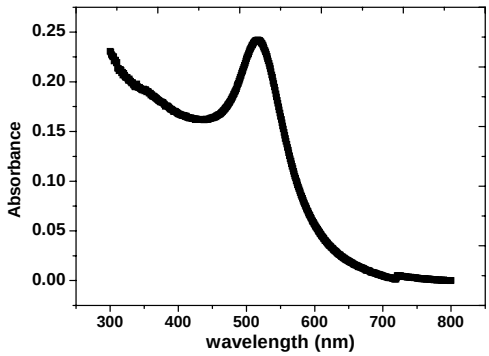


Figure S-3. UV-Vis absorption spectrum of Uncapped Au NPs

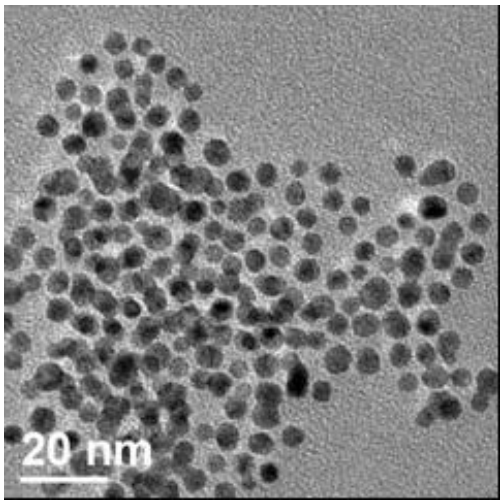


Figure S-4. TEM picture of Au nanoparticles

Preparation of CdS:Mn NCs film. Before the surface modification, the GCE was polished in sequential order with 1.0, 0.5 and 0.3μm alumina. The GCE was thoroughly washed with water, sonicated in ethanol, and finally dried in air. 10 μL of CdS:Mn NCs solution was drop-cast on the pretreated GCE and dried in air at room temperature, as a result, even NCs film was obtained. The CdS:Mn NCs modified GCE was stored in 0.1M NaCl + 0.1M Tris-HCl buffer (pH 7.4) for characterization and further modification.

Preparation of hairpin DNA probe/MCH/Au NP composite. In typical experiments, 50 μL of 0.85 μM hairpin DNA probe in 0.1M NaCl +0.1M Tris-HCl buffer was added into 500 μL Au colloidal solution containing 0.1M NaCl, followed by addition of 5 μL of 0.73 M MCH in 0.1M NaCl +0.1M Tris-HCl buffer. The resulting colloidal solution was kept in refrigerator at 4ºC for 2 h. The use of MCH is believed to remove nonspecifically adsorbed and some of the chemically attached DNA from AuNPs surface, which helps to improve subsequent hybridization efficiency.

Assembling of hairpin DNA probe/MCH/Au NP composites to CdS:Mn NCs film on GCE. Typically, the beforehand prepared CdS:Mn NCs film on GCE was immersed in 1.0 mL 0.1M NaCl + 0.1 M tris-HCl buffer containing 3 mM MPA for 5 h at 4ºC. After rinsed thoroughly with ultra-pure water and tris-HCl buffer, the terminal carboxylic acid groups of the MPA/CdS:Mn/GCE were activated by immersion in 1.0 mL of 0.1M 1-methylimidazol aqueous solution (pH 7.4) containing 20 mg EDC and 10 mg NHS for 2 h at 4ºC. The linker/MPA/CdS:Mn NCs/GCE was rinsed with 0.1M tris-HCl buffer (pH7.4) to wash off the excess EDC and NHS. Finally, the

resulting linker/MPA/CdS:Mn NCs/GC electrode was soaked in the stable colloidal solution of hairpin DNA probe/MCH/Au NP composites (100 µL) for 24 h at 4ºC. The as-prepared DNA biosensor was washed thoroughly with 0.1 M NaCl+0.1 M tris-HCl buffer (pH 7.4) and stored in the same buffer for 24 h to remove the unlinked hairpin DNA probe /Au NP composites before DNA hybridization test.

DNA hybridization. The hairpin DNA probe/MCH/Au NP/MPA/CdS:Mn NCs/GCE was incubated in 70 µL of 0.1M NaCl + 0.1M tris-HCl buffer (pH 7.4) containing different concentrations of oligonucleotide at room temperature for 1 h. Subsequently, the electrode was washed thoroughly with the same buffer to remove unhybridized oligonucleotide followed by the measurement of ECL.

Characterization of CdS:Mn film on GCE

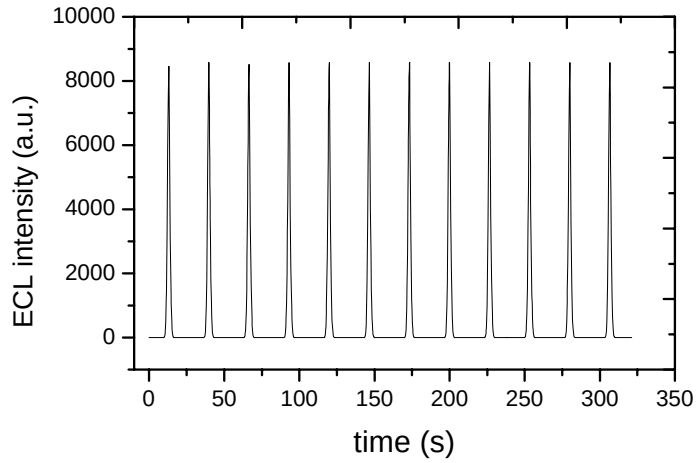


Figure S-5. ECL emission from MPA/CdS:Mn NCs on GCE in 0.05M $K_2S_2O_8$+0.1M PBS (pH 8.3) under continuous cyclic potential scan for 12 cycles. Scan rate, 100 mV s-1.

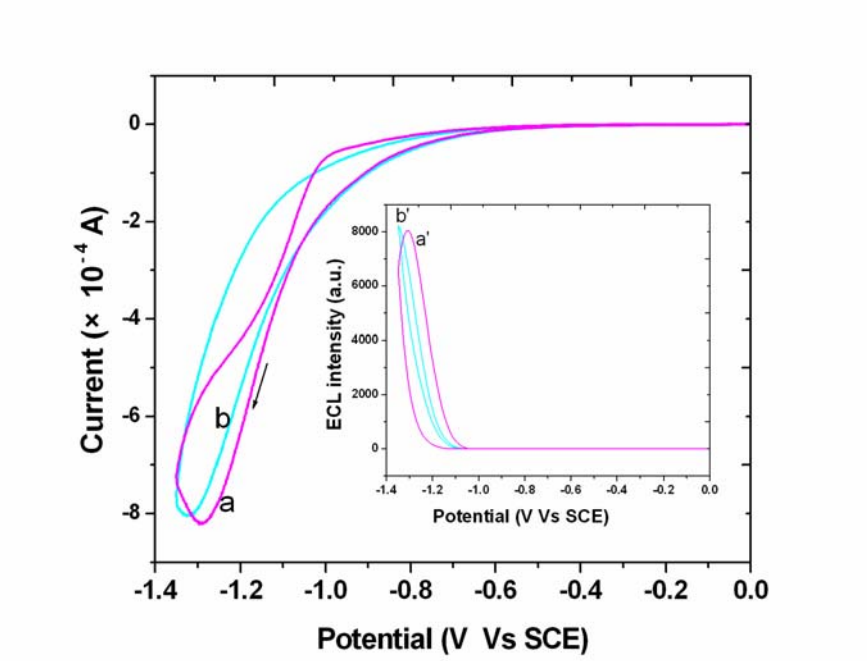


Figure S-6. Cyclic voltammograms of 0.05M $K_2S_2O_8$ on (a) CdS:Mn NCs modified GCE and (b) CdS:Mn NCs modified GCE after the self-assembly of MPA; inset: (a') corresponding cyclic ECL curve of a; (b') corresponding cyclic ECL curve of b; ECL detection buffer: 0.05M $K_2S_2O_8$ +0.1M PBS (pH 8.3). Scan rate, 100 mV s-1.

ECL Spectrum of Undoped CdS NCs Film

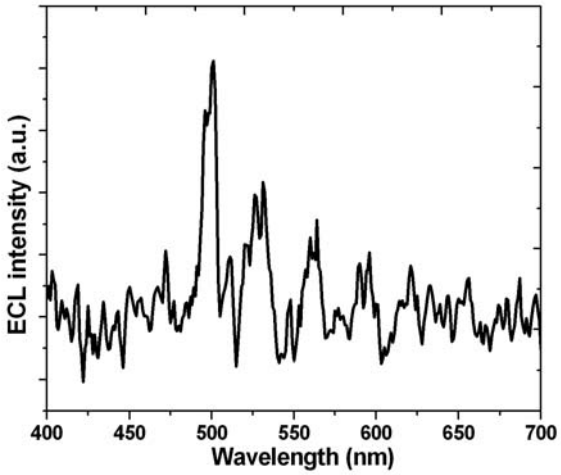


Fig S-7. ECL spectrum of the undoped CdS NCs film in 0.05 M $K_2S_2O_8$ +0.1M PBS (pH 8.3) obtained by stepping potential between 0 and -1.5V

Reference

S1 Anand Gole and Catherine J. Murphy. *Chem. Mater.* 2004, **16**, 3633-3640.